Efficient transformation of germinating Neurospora conidia using total nuclear DNA fragments.

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Abstract
Efficient transformation of germinating Neurospora conidia using total nuclear DNA fragments.

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which were centrifuged at 4°C for 45 min at 58,300 g (w, 6.6 cm) using a DuPont-Sorvall OTD-2 ultracentrifuge. Owing to the size heterogeneity among Percoll particles, they sediment (and diffuse) at different rates in a gravitational field, thereby creating a density gradient. The biological material in the gradient, in this case nuclei, bands isopycnically, so that the sample particles reach a position where their densities and that of the surrounding Percoll medium are equal. As is the case with isopycnic separation using cesium chloride gradients, a fixed angle rotor has advantage over a swinging bucket rotor since with fixed angle rotors reorientation of the tube contents does not occur to alter the final separation of the zones and there is better resolution of the experimental materials since they are banded over a larger cross sectional area.

A range of starting densities of Percoll from 1.05 to 1.12 gm⁻¹ were tested in separate experiments to determine the most useful for banding Neurospora nuclei. After each experiment the tube contents were fractionated into 12 fractions, and their refractive index determined with an A/O Refractometer. The results showed that the centrifugation generated adequate Percoll gradients. The nuclei banded to one region of the gradient but the band was not homogeneous: the upper part was relatively disperse, the center was dense and homogeneous, and the lower part exhibited some clumps. Based on refractive index measurements, the density of the nuclei was determined to be 1.078 gm⁻¹. The nuclei may be recovered by centrifuging gradient fractions containing nuclei for 2 h at 100,000 g (w, 6 cm) in a swinging bucket rotor. Under these conditions, the silica particles pellet and the nuclei remain above the gel formed. The nuclei may then be pelleted from the supernatant liquid by centrifugation for 20 min at 5,000 g (w, 6 cm).

In conclusion, the results indicate that Percoll is an effective alternative to Ludox for the purification of Neurospora nuclei from crude nuclear preparations. The absence of large osmotic effects such as is observed with other gradient materials has allowed the density of wild type nuclei to be determined. Finally, although RNA extracted from crude nuclei includes high molecular weight species that are presumptive precursors to mature rRNA (K. Talbot 1980 Baccalaurate Thesis, Reed College), studies of pre-rRNA processing in the nucleolus will be greatly facilitated now that pure nuclei can be obtained. (Supported by NIH grants GM22488, GM22489).

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Wootton, J.C., M.J. Fraser, and A.J. Baron. Efficient transformation of germinating Neurospora conidia using total nuclear DNA fragments.

We have developed a simple, reliable transformation method that does not require enzymic digestion of cell walls. This method exploits the tendency of inl and os strains to form swellings resembling sphaeroplasts on germ tubes when macroconidia are germinated in the absence of inositol in media of high osmolality. Protophoretic transformants have been obtained from several auxotrophs of low or zero spontaneous reversion frequency, including the well defined deletion strain am Donor DNA was prepared from nuclear pellets by essentially the method of Hautala et al. (1977 J. Bacteriol. 130:704) and was usually used as linear fragments of average molecular weight at 2 x 10⁻³ without further shearing. DNA sheared to an average molecular weight of 5 x 10⁶ gave similar results. Restriction fragments and single stranded DNA (the latter obtained by melting and rapid cooling of unsheared preparations of nuclear DNA as above) have also been used successfully. Detailed results will be reported elsewhere (submitted for publication).

In a typical experiment, 2 x 10⁸ conidia of the recipient strain were germinated in 1 ml of Vogel's minimal medium containing 20% sucrose (w/v) and all required supplements except inositol for 3 to 5 hours at 30°C on an orbital shaker. When the majority of germ tubes showed swellings indicating weakened cell walls, the conidia were harvested by centrifugation, washed 3 times with Vogel's minimal medium containing 1M-mannitol and suspended in 0.4 ml of this medium. Donor DNA (1 to 5 μg) was precondensed by mixing with 0.1 ml of 500 mM-CaCl₂ and added to the suspension of germinated conidia. Incubation was continued for 1 h at 30°C on an orbital shaker. Treated suspensions were pelleted through 10% sorbose (w/v) or 0.8M-mannitol for plating (either by spreading or in thin layers of soft agar) on sorbose (w/v) or 0.8M-mannitol medium containing 0.6M-mannitol and supplements as appropriate for selection of protrophs or viability measurements. Viability of partially sphaeroplasted conidia obtained by this method is usually greater than 90% Control treatments were also included using DNA prepared from the recipient, DNase-digested DNA, and CaCl₂ alone.

Transformation frequencies obtained by this method using inl recipients ranged from 0.5 to 12.3 transformants per μg of DNA (1.5 x 10⁻⁷ to 4.2 x 10⁻⁷ per viable recipient conidium), with a mode around 5 transformants per μg of DNA (1.5 x 10⁻⁶ per viable conidium), os recipients have given similar results. For comparison, the same recipient strains have been used in transformations employing the mycelial fragment method described by Mshra and Tatum (1973, Proc. Natl. Acad. Sci. 70:3875) and a protoplasting method similar to that of Hinnen et al. (1978 Proc. Natl. Acad. Sci. 75:1929). Both of these methods gave transformation frequencies in the 0.04 to 0.12 transformants per μg of DNA (0.8 x 10⁻⁷ to 2.9 x 10⁻⁷ per viable fragment or protoplast plated), approximately 50-fold lower frequencies than those obtained by our method using germinating conidia.

The choice of inl strains may be important. Best results have been obtained with recipients carrying inl 37401 (FGSC 2145) and inl R233 crossed from an isolate in our collection originally obtained from R. Gross. However, the properties of inl R233 stocks changed after 3 backcrosses into STA4 background, and very low transformation frequencies were subsequently obtained. Presumably the kinetics of the lytic processes involved in inositol-less death are important for successful partial sphaeroplasting and may be influenced by genetic background. Department of Genetics, University of Leeds, Leeds LS2 9JT, United Kingdom.